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CAATCAGCAGACAGCAGTGGTACTTATGAGGTA33

CLAIMS:

What is claimed is:

1. A human monoclonal antibody which specifically binds to a surface antigen of a stomach cancer cell MKN 45, said antibody belonging to IgG class, and said antibody having a variable region of the heavy chain which is the amino acid sequence shown in SEQ ID No. 5 and a variable region of the light chain which is the amino acid sequence shown in SEQ ID No. 6.

2. A F(ab').sub.2 fragment of the human monoclonal antibody of claim 1.

3. A Fab' fragment of the human monoclonal antibody of claim 1.

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L1: Entry 3 of 3 File: USPT Jun 16, 1998

US-PAT-NO: 5767246
DOCUMENT-IDENTIFIER: US 5767246 A

TITLE: Human monoclonal antibody specifically binding to surface antigen of cancer cell membrane

DATE-ISSUED: June 16, 1998

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PRIOR-ART-DISCLOSED:

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Search Selected | Search ALL

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ART-UNIT: 186

PRIMARY-EXAMINER: Schwadron; Ronald B.

ABSTRACT:

A human monoclonal antibody specifically binding to a surface antigen of cancer cell membrane, an isolated DNA encoding the antibody, and a hybridoma producing the antibody. An anti-cancer formulation comprising the monoclonal antibody bonded to the surface of a liposome enclosing an anti-cancer agent or toxin is also provided.

3 Claims, 5 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 4

BRIEF SUMMARY:

1 The present invention relates to a novel human monoclonal antibody useful for diagnosis and therapy of cancer, an isolated DNA encoding the monoclonal antibody, and a hybridoma producing the antibody. The present invention also relates to an anti-cancer formulation comprising the antibody bonded to a liposome which contains an anti-cancer agent.

2 There has been no anti-cancer formulation thus far, which is sufficiently effective for the treatment of solid cancer. On the other hand, there has long

existed an idea called "targeting" in which a therapeutical agent is concentrated at a tissue or an organ to be treated in order to maximize the therapeutical effect of the agent. Accordingly, it has been expected that focusing an anti-cancer agent at a cancer tissue by means of "targeting" may allow a therapy of the solid cancer. A number of trials to concentrate an anti-cancer agent or a toxin at a cancer tissue were made since a method for production of mouse monoclonal antibodies in large quantities has been established by Milstein and Kohler (Nature, 1975), and some of them were successful.

3 Thus far, binding of an antibody to a therapeutic agent has been accomplished by directly binding an antibody to a chemically-modified therapeutic agent, or indirectly binding them via a water-soluble polymer such as dextran. These methods, however, have drawbacks in that the amount of a therapeutic agent capable of binding to one antibody molecule is very limited, and in that chemical modification of a therapeutic agent often causes lowering of the therapeutical activity. As one of the countermeasures to overcome the drawbacks, there was proposed a new delivery system which consists of an antibody bonded to the surface of a liposome in which a therapeutic agent is encapsulated, and many favorable results were reported (Kono et al, Cancer Research 47 4471, 1987; Hashimoto et al, Japanese Patent Publication (unexamined) No. 134012/1988).

4 However, mouse monoclonal antibodies have a limited clinical use and continued administration thereof is impossible from a practical point of view due to side effects such as anaphylaxis caused by immune response (See A. Lo Bugli et al, Proc. Natl. Acad. Sci. U.S.A., 86 4220, 1989). Accordingly, human monoclonal antibodies rather than mouse monoclonal antibodies are preferable for the purpose of clinical use. However, preparation of human monoclonal antibodies which adequately react with cancer cells has long been considered very difficult because of the reasons that it is very difficult to conduct passive immunity for the purpose of obtaining human B cells which produce a desired antibody, and that any efficient methodology which allows infinite reproduction of antibody-producing cells has not been established yet.

5 In such a situation as mentioned above, the inventors of the present invention have made extensive study for the purpose of obtaining a human monoclonal antibody which permits "targeting therapy" on cancer tissue or organ with the help of anti-cancer agents or toxins, and they have succeeded in preparing a hybridoma capable of producing a novel human monoclonal antibody, the antigen to which exists on the surface of cell membrane of cancer cells. They also have succeeded in preparing a therapeutical formulation useful for "targeting therapy" of cancer, by binding the monoclonal antibody of the invention to a liposome in which an anti-cancer agent is encapsulated. The present invention is based on these findings.

6 Thus, the present invention provides a human monoclonal antibody specific to an antigen existing on the surface of a cancer cell membrane, said monoclonal antibody being produced by a fused cell between a lymphocyte derived from cancer patient and a mouse myeloma cell. The invention further provides an isolated gene encoding the antibody, a hybridoma producing the antibody, and an anti-cancer formulation containing the antibody.

7 The human monoclonal antibodies of the present invention contain, in the variable region of the heavy chain, the amino acid sequences shown, for instance, in Sequence Listing Nos. 13, 14, and 15. More specifically, the monoclonal antibodies of the invention include, among others, those in which the variable region of the heavy chain comprises the amino acid sequences shown in Sequence Listing Nos. 16, 17, and 18, and the variable region of the light chain comprises the amino acid sequences shown in Sequence Listing Nos. 19, 20, and 21, and those in which the variable region of the heavy chain comprises the amino acid sequences given in Sequence Listing Nos. 22, 23, and 24, and the variable region of the light chain comprises the amino acid sequences given in Sequence Listing Nos. 25, 26, and 27.

8 The monoclonal antibodies of the invention include any variants of the above-mentioned specific antibodies, which are obtainable by making insertion, deletion, substitution and/or addition of one or more amino acid residues to the amino acid sequences of the above-identified antibodies with the limitations that such modification must not adversely affect the reactivity of the antibodies against the antigens. The present invention will be more detailed below.

DRAWING DESCRIPTION:

In the accompanying drawings;

FIG. 1 schematically shows the construction of vector pHCRD.

FIG. 2 schematically shows the construction of vector pHCR(.DELTA.E)'H.

FIG. 3 shows reactivity of antibody 1-3-1 to colon cancer cell line C-1.

FIG. 4 shows reactivity of antibody 1-3-1 to gastric cancer cell line MKN45.

FIG. 5 shows anti-cancer effects of adriamycin-containing and PEG-modified liposome bonded to antibody CAR on the cancer transplanted to nude mouse.

DETAILED DESCRIPTION:

1 The hybridoma producing a human monoclonal antibody of the invention is prepared according to the method described by A. Imam (Cancer Research 48 263, 1988). Thus, lymphocytes which have been isolated from extracted lymph node associated with cancer are fused with mouse myeloma cells in the presence of polyethylene glycol. Hybridomas thus obtained are screened by means of enzyme immunoassay using various cancer cell line fixed with paraformaldehyde, and hybridomas capable of producing antibodies are obtained and cultured. From supernatant of the resulting culture, monoclonal antibodies are isolated and purified according to a conventional method such as disclosed by R. J. Duhamel (J. Immunol. Methods 31 211, 1979).

2 The purified monoclonal antibody is labelled with a fluorescent substance and examined about its reactivity with living cancer cells and normal cells such as erythrocytes and leucocytes using Flow Cytometry. Hybridoma producing an antibody which reacts with the living cells but not with normal cells are selected. Alternatively, the reactivity of antibodies to cancer cells isolated from cancer tissue of a patient is compared with the reactivity to normal cells derived from non-cancer segment of the same organ, and a hybridoma producing an antibody which reacts with the cancer cell and does not react, or reacts as moderately as an antibody derived from normal volunteer, with normal cells, is selected.

3 A base sequence of a DNA encoding a human monoclonal antibody produced by the hybridoma selected above can be determined in the following manner.

4 In accordance with Casare et al method (DNA 2 123, 1983), mRNAs are separated from the antibody-producing hybridoma cells, using guanidine thiocyanate-lithium chloride, and cDNA library is prepared by the use of oligo (dT) primer. The cDNAs thus obtained are then subjected to (dG) tailing. Consensus sequence between poly C capable of hybridizing with the dG tail obtained above and an already available human gene encoding heavy or light chain of human antibodies is used as a probe for amplification of the antibody-encoding cDNA by means of PCR. The terminal of the amplified DNA is made blunt. The DNA separated from an electrophoresis gel is inserted to a cloning vector such as pUC119, and the base sequence of the DNA is determined

by Sanger et al dideoxy method (Proc. Natl. Acad. Sci. U.S.A. 74 5463, 1977).

5 Preferable antibodies of the present invention are those in which the variable region of the heavy chain comprises the amino acid sequences shown in Sequence Listing Nos. 13, 14, and 15. Specific examples of preferred antibodies are, among others, those in which the variable region of the heavy chain comprises the amino acid sequences shown in Sequence Listing Nos. 16, 17, and 18, and the variable region of the light chain comprises the amino acid sequences shown in Sequence Listing Nos. 19, 20, and 21, and those in which the variable region of the heavy chain comprises the amino acid sequences shown in Sequence Listing Nos. 22, 23, and 24, and the variable region of the light chain comprises the amino acid sequences shown in Sequence Listing Nos. 25, 26, and 27.

6 The above-noted amino acid sequences in Sequence Listing Nos. 13, 14, and 15; 16, 17, and 18; and 22, 23, and 24 are called "hyper variable region" in variable region of the heavy chain. Likewise, the amino acid sequences in Sequence Listing Nos. 19, 20, and 21; 25, 26, and 27 are called "hyper variable region" in variable region of the light chain. These regions are responsible for the specificity of the antibody and determinative to binding affinity between the antibody and the antigenic determinant. Accordingly, the variable region of the heavy chain in the antibodies of the invention can have various amino acid sequences derived from different antibodies so far as it comprises the above-mentioned hyper variable regions.

7 The most preferred monoclonal antibodies of the invention are those in which the variable regions of the heavy and light chains are represented by the amino acid sequences of Sequence Listing Nos. 4 and 6 respectively, and also 11 and 12 respectively. The DNA sequences encoding constant regions of the heavy and light chains are the same as those disclosed in Nucleic Acids Research 14 1779, 1986, The Journal of Biological Chemistry 257, 1516, 1982 and Cell 22, 197, 1980, respectively.

8 The monoclonal antibody of the invention may be prepared by culturing the hybridoma producing the antibody of the invention in eRDF or RPMI1640 medium containing fetal bovine serum. Alternatively, it may also be prepared by connecting the DNAs having the base sequences in Sequence Listing No. 3, 4, 9 and No. 10, which encode variable regions of heavy and light chains respectively, with known DNAs encoding the constant regions as mentioned above to obtain a pair of genes encoding the monoclonal antibody of the invention, inserting the genes into one of various known expression vectors, transforming an appropriate host cell such as CHO cell with the expression vectors, and culturing the resultant transformant. As expression vectors to be used in animal cells, there may conveniently used a combination of PMCF (.DELTA.E)-H and pKCHO which may be constructed in the manner as shown in FIGS. 1 and 2 starting from pMCFH2 disclosed by Nishina (Nature 317 814, 1985). In more detail, a gene encoding the heavy chain, to which a HindIII restriction site has been added, is inserted into plasmid pMCF (.DELTA.E)-H at the HindIII site, and a selective marker such as DHFR gene is inserted into the plasmid at SalI site. On the other hand, a gene encoding the light chain, to both ends of which EcoRI restriction site has been added, is inserted into plasmid pKCHO at EcoRI site, and then the DHFR gene is also inserted into the plasmid at SalI site. Both of the plasmids obtained above are incorporated into a host cell such as CHO cell (Urlaub G. & Chasin L. A., Proc. Natl. Acad. Sci. U.S.A., 77 4216, 1980) by means of calcium phosphate method. The resultant transformant is cultured in .alpha.MEM medium containing no nucleotide, and grown cells are subjected to further selection for antibody-producing clones. The antibody of the invention can be obtained and purified by culturing the selected clone, adsorbing the resulting supernatant to a column filled with Protein A supported by cerulofine or agarose, and eluting the antibody from the column.

9 A liposome used for the preparation of the anti-cancer formulation of the invention is composed of two lipid layers. The lipid layer may be of monolayer or multiple layers. Constituents of the liposome are phosphatidylcholine, cholesterol, phosphatidylethanolamine, etc. Phosphatidic acid, which provides

the liposome with electric charge, may also be added. The amounts of these constituents used for the production of the liposome are, for instance, 0.3-1 mol, preferably 0.4-0.6 mol of cholesterol, 0.01-0.2 mol, preferably 0.02-0.1 mol of phosphatidylethanolamine, 0.9-1.4 mol, preferably 0-1.15 mol of phosphatidic acid per 1 mol of phosphatidylcholine.

10 The liposome used in the present invention may be prepared by conventional methods. For example, a mixture of the above-mentioned lipids, from which the solvents have been removed, is emulsified by the use of a homogenizer, lyophilized, and melted to obtain multilamera liposome. Adjustment of particle size of the resultant liposomes may be conducted by ultrasonication, high-speed homogenization, or pressure filtration through a membrane having uniform pore size (Hope M. J. et al., Biochimica et Biophysica Acta 812 55, 1985). Preferable particle size of the liposomes are between 30 nm and 200 nm.

11 Anti-cancer agents encapsulated in the liposome includes carcinostatin agents such as adriamycin, daunomycin, mitomycin, cisplatin, vincristine, epirubicin, methotrexate, 5 Fu, and aclacinomycin, toxins such as ricin A and diphtheria toxin, and antisense RNA. Encapsulation of anti-cancer agent into liposome is accomplished by hydration of the lipids with an aqueous solution of the anti-cancer agent. Adriamycin, daunomycin, and epirubicin may be encapsulated into a liposome by means of remote loading method taking advantage of pH gradient (Lawrence D. M. et al., Cancer Research 48 2621, 1988).

12 Binding of a monoclonal antibody to the surface of the liposome mentioned above may be accomplished by the formation of cross-linkage between phosphatidylethanolamine and the antibody using glutaraldehyde. However, preferred method is that a thiolated antibody is allowed to react with a liposome comprising a lipid into which a maleimide group has been incorporated. Remaining maleimide groups on the surface of the liposome may be further reacted with a compound containing thiolated polyalkyleneglycol moiety, thereby the surface of the liposome is modified.

13 Thiolation of an antibody may be conducted by the use of N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), which is usually used for thiolation of protein, iminothiolane, or mercaptoalkylimidate. Alternatively, a dithiol group intrinsic to an antibody may be reduced to form a thiol group. The latter is preferred from the view point of keeping antibody's function. Another method to provide an antibody with a thiol group is that an antibody is treated with an enzyme such as pepsin to form F(ab)'.sub.2, which is then reduced with dithiothreitol (DTT) to form Fab', which gives one to three thiol groups.

14 The binding of the thiolated antibody to the maleimide group-containing liposome may be accomplished by reacting them in a neutral buffer solution at pH 6.5-7.5 for 2-10 hours.

15 The anti-cancer formulation of the present invention may be prepared by means of conventional methods such as dehydration method (Japanese Patent Publication No. 58932/1988) and lyophilization method (Japanese Patent Publication No. 811/1989).

16 The anti-cancer formulation of the invention may be administered intravascularly, peritoneally, or locally. Dosage of the formulation varies depending on the nature of particular anti-cancer agent encapsulated into the liposome. When the agent is adriamycin, the dosage is the one corresponding to adriamycin 50 mg or less/kg body weight, preferably 10 mg or less/kg, more preferably 5 mg or less/kg.

17 The following detailed examples are presented by way of illustration of certain specific embodiments of the present invention.

18 EXAMPLE 1

19 Establishment of Hybridoma Producing Human Monoclonal Antibody GAH

20 Hybridoma producing human monoclonal antibody GAH was established by cell fusion between lymphocytes derived from a lymph node associated with cancer tissue of a patient and mouse myeloma cells.

21 (1) Preparation of Lymphocytes

22 Cancer-associated lymph node extracted from a patient suffering from colon cancer was cut up into fine pieces with scissors and scalpel, and cells were dispersed using a stainless net in Culture Medium A (eRDF (Kyokuto Seiyaku Kogyo)+50 .mu.g/ml gentamicin sulfate). The resultant cell suspension was centrifuged at 1000 rpm for 10 minutes and the supernatant was discarded. The residue was suspended in fresh Culture Medium A, and the suspension was centrifuged again to obtain 2.6.times.10.sup.7 cells.

23 (2) Cell Fusion

24 The lymphocyte cells obtained above were subjected to cell fusion with mouse myeloma cells (1.times.10.sup.7) in the presence of polyethyleneglycol (Boehringer-Mannheim) according to a conventional method. The fused cells were suspended into Culture Medium A added with 10 .mu.M hypoxanthine, 7.34 .mu.M aminopterin, 1.6 .mu.M thymidine, and 10% fetal calf serum (FCS), said medium being referred to as HAT addition medium hereinafter, so that the density of the lymphocytes may be 5.4.times.10.sup.5 / ml. The suspension was plated on 96 well plates at 100 .mu.l/well and cultured at 37.degree. C. in a 5%.sub.2 incubator. Half of the culture medium was substituted with HAT addition medium from time to time and the cultivation was continued until hybridoma's colonies appeared. The hybridoma's colonies were observed in all of the wells. The supernatant of the culture in each well was tested on the reactivity to several established cancer cell lines such as colon cancer cell line C-1 (Sato et al, Ipakuhoshuri (Progress of Medicine) 96 676, 1976, obtained from Med. Ex. Seibutsu Kenkyusho (Institute of Immunized Organisms)), and stomach cancer cell line MKN45 (Saito et al, Gan to Kagaku Ryoho (Cancer and Chemotherapy) 5 33, 1978, obtained from above-noted Institute) according to the method described in Experiment 1. Positive wells were 7.5% (35 wells) against C-1 and 4.6% (12 wells) against MKN45, and 6 wells showed positive reaction to both strains. Cloning of hybridomas was conducted using the wells which showed positive reaction to both lines. The cloning was conducted three times by means of limiting dilution method, and hybridoma clone GAH was established.

25 EXAMPLE 2

26 Purification and Labeling of Monoclonal Antibody GAH

27 (1) Culture of Hybridoma GAH and Purification of Monoclonal Antibody GAH

28 Fetal calf serum was passed through a Protein A-agarose (BepiGen), thereby substances absorbed to the column was removed from the serum. For culture of hybridoma GAH, eRDF culture medium (Kyokuto Seiyaku) to which 5% of the above serum had been added was used. The culture of hybridoma GAH was then charged into a Protein A-agarose column, and adsorbed antibody was then eluted out to obtain purified antibody. The use of the above-noted serum allowed to obtain pure antibody GAH free from other antibodies of serum origin and substances absorbed to Protein A-agarose. The antibody GAH was confirmed to be a pure IgG by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

29 (2) Fluorescent Labeling of Antibody GAH

30 The purified antibody GAH was labeled by fluorescein isothiocyanate (FITC) according to the method of Coons A. H. Thus, the antibody was dialyzed against a carbonate buffer solution (pH 9.5) and reacted with FITC solution. The

labeled antibody was separated from free FITC by gel filtration. Absorbance of fractions containing labeled antibody was measured at OD.sub.280 nm and OD.sub.495 nm and labeling degree was determined. The binding molar ratio of the antibody and FITC (F/P ratio) was 0.93.

31 EXPERIMENT 1

32 Study on Reactivity of Human Monoclonal Antibody against Cancer Cell Lines

33 1) Cancer Cell Lines and Preservation Thereof

34 Colon cancer cell line C-1 and stomach cancer cell line MKN45 were used as human cancer cell lines. The cells were preserved and grown at 37.degree. C. under 5% CO.sub.2 conditions using Culture Medium B (eRDF medium containing 10% FCS).

35 2) Study on Reactivity to Cancer Cell Lines

36 a. Determination of reactivity against solid cancer cell lines

37 Cancer cells were cultured until monolayer in a 96 well plate for 3 or 4 days. After removal of culture supernatant, the plate was washed twice by 10 mM phosphate buffer (pH 7.4) and 0.15M NaCl solution (PBS), and 2% paraformaldehyde fixation was conducted at room temperature for 20 minutes. After washing 3 times with PBS, PBS solution containing 3% BSA (bovine serum albumin) was added to wells (200.mu.l/well), and the plate was kept 37.degree. C. for 2 hours to complete blocking. The plate was washed 3 times with PBS, and 50 .mu.l of culture supernatant of hybridoma was added thereto. After two hour reaction at 37.degree. C., the plate was washed 3 times with PBS and 50 .mu.l of alkaliphosphatase conjugated goat antibody to human antibody (1000 dilution, Tago) was added. Following one hour reaction at 37.degree. C., the plate was washed 3 times with PBS and added with 1.0M carbonate buffer--1 mM MgCl (pH 9.8) containing 25 mM p-nitrophenyl phosphate at ratio of 50 .mu.l/well and allowed to react at room temperature for one hour to overnight. Absorbance at 405 nm was measured with micro-plate photometer (Colinar). Reactivity was determined according to the method described in Example 1 (2). Cloning from the wells in which positive reaction against cultured cancer cell lines C-1 and MKN45 has been observed gave hybridoma GAH. Purified antibody from culture supernatant of GAH showed the same reactivity.

38 b. Reactivity to living cancer cells

39 Cancer cells were cultured in a flask or Petri dish and culture supernatant was discarded. To the residue was added a PBS solution containing 0.02% EDTA, and the mixture was left to stand at room temperature for 10 minutes allowing the cells to float. The cells were washed with Culture Medium B by centrifugation and suspended in healthy human serum containing the fluorescent-labeled antibody GAH (final concentration: 50 .mu.g/ml) obtained in Example 2 (2) so that cell density of about 1.times.10.sup.6 /300 .mu.l may be obtained, and the suspension was allowed to react at 0.degree. C. for 60 minutes. The suspension was centrifuged at 1000 rpm for 3 minutes and the supernatant was discarded. The remaining cells were suspended in 1 ml of PBS, washed by centrifugation, and resuspended in 300 .mu.l of PBS containing 10 .mu.g/ml of propidium iodide (PI). The suspension was subjected to the observation by flow cytometer (FCM), FACS440 (Becton Dickinson), in order to determine the magnitude of fluorescence (FITC and PI) bonded to particular cell. Dead cells having PI fluorescence could be removed because the dead cells took in PI in the nucleic acids and emitted PI fluorescence. Markers having five standard amounts of fluorescence (quantitative kit: Ortho Diagnostic Systems) were subjected to FCM under the same conditioned as above. Based on the markers, average binding amount of FITC per cell was calculated. On the basis of the average binding amount and F/P ratio of labeled antibody, an average number of antibodies bonded to one living cell was determined. The results are shown in Table 1.

TABLE 1

Cancer Cell Strain	Antibody GAH	Control IgG
MKN45	3.5 .times. 10.sup.4	.15 .times. 10.sup.4
G-1	0.6 .times. 10.sup.4	0.1 .times. 10.sup.4

40 When compared with IgG derived from healthy human serum, which was labeled by fluorescence in the same manner as GAH and used as a control, about 6-23 times larger amount of antibody GAH has bonded to stomach and colon cancer cells.

41 EXPERIMENT 2

42 Reactivity of Human Monoclonal Antibody GAH to Blood Cells

43 Erythrocytes were separated from peripheral blood taken from 7 healthy volunteers and 3 patients suffering from cancer according to Kinoshita's method (Separation of Erythrocytes; New Edition of Nippon Ketsuekigaku Zensho 13 800, 1979).

44 Leukocytes were obtained in the following manner: Peripheral blood was drawn from healthy volunteers with addition of heparin. 2 ml of 6% dextran-physiological saline was added and mixed to 10 ml of the blood. The mixture was left to stand at room temperature for 30 minutes to give a plasma layer, which was then separated and centrifuged at 1500 rpm for 5 minutes to obtain leukocytes.

45 Reactivities of the monoclonal antibody of the invention to these blood cells were determined by means of FCM in the same manner as in the living cancer cells except that PI was not added. In this connection, the leukocytes were divided into lymphocyte (major leukocyte cell), granulocyte, monocyte, and platelet, based on front and side light scattering in FCM (Bio/Technology 3 907, 1985), and reactivities to respective cells were separately determined. The test results were shown in Table 2.

TABLE 2

Cells	Antibody GAH	Control IgG
Leukocyte		
lymphocyte	negative	negative
granulocyte	0.49 .times. 10.sup.4 *	0.48 .times. 10.sup.4 *
monocyte	0.41 .times. 10.sup.4 *	0.43 .times. 10.sup.4 *
platelet	negative	negative
Erythrocyte	negative	negative

*Average number of antibodies bonded per cell

46 Antibody GAH showed no reaction to erythrocyte and lymphocyte, while the

reactivity to granulocyte and monocyte was the same level as the reactivity to control IgG likewise in Experiment 1.

47 EXPERIMENT 3

48 Reactivity of Human Monoclonal Antibody GAH to Cells Derived from Fresh Cancer Tissue and Non-Cancer Tissue

49 In order to study a binding specificity of antibody GAH to cancer cells, normal cells were simultaneously isolated from fresh tissue belonging to the same organ of the same patient from which cancer cells were obtained, and reactivities of antibody GAH to respective cells were determined. Isolation of cells from the tissue was conducted according to Tokita's method (Ganno Rinsho (Cancer in Clinic) 32 1803, 1986).

50 Thus, the tissue extracted was placed on Teflon sheet spreaded on a rubber plate, cut with a razor into fine pieces, and transferred onto a 1 mm stainless meshes. The meshes was shaken in a Petri dish full of a culture medium to obtain the medium containing small cell aggregates which passed through the meshes. The medium was centrifuged at 1000 rpm, and floating fats and suspending necrotic cells were discarded. This centrifugation was repeated several times. The cell aggregates were subjected to pumping by means of a syringe with Cateran needle of 23 gauge to disperse the cells. The reactivity to the cells thus obtained was determined by FCM in the same manner as in the living cancer cells. The test results are shown in Table 3.

TABLE 3

Antibody	Colon Cancer Cells	Colon Non-cancer Cells	Stomach Cancer Cells	Stomach Non-cancer Cells
GAH	1.1 .times. 10.sup.4	0.03 .times. 10.sup.4	189 .times. 10.sup.4	4.6 .times. 10.sup.4
Control IgG	0.15 .times. 10.sup.4	0.04 .times. 10.sup.4	3.8 .times. 10.sup.4	4.3 .times. 10.sup.4

*Average number of antibodies bonded per cell

51 The average number of GAH antibodies bonded to cancer cells is remarkably higher than that in the non-cancer cells. In addition, the number of antibodies bonded to cancer cells was 81 times greater than that in the control IgG in stomach cancer, and 7 times greater in colon cancer. These results indicate that antibody GAH recognizes an antigen dominantly expressed on the surface of cell membrane of cancer cells.

52 EXAMPLE 3

53 (1) Determination of Subclass of Light Chain of Monoclonal Antibody GAH

54 Antibody GAH obtained in Example 2 (1) was subjected to SDS-PAGE in the reduced form. Heavy chain and light chain separately electrophorated were blotted on a transmembrane (Polyvinylidene-dilluoride, Millipore). The membrane was blocked with 5% BSA solution and allowed to react with a goat antibody to human .kappa. or .lambda. chain, which was combined with peroxidase (Capel). After washing, a

0.05% (w/v) 4-chloronaphthol solution containing 0.015% H.sub.2 O.sub.2 was allowed to react thereto as a substrate. The light chain of antibody GAH reacted with anti-human .kappa. chain antibody, which was detected through the appearance of colored band. This revealed that the light chain was .kappa. chain.

55 2) Preparation of Gene Encoding Monoclonal Antibody GAH

56 a. Preparation of cDNA encoding antibody GAH by means of polymerase chain reaction (PCR)

57 According to the method detailed below, poly(A)-containing RNAs were prepared from antibody GAH-producing hybridoma obtained in Example 1 2) using guanidine thiocyanate-lithium chloride method (DNA 7 321, 1988).

58 The hybridoma cells (1.times.10.sup.7) were solubilized in a solution (7.5 ml) comprising 3M guanidine thiocyanate, 17 mM EDTA, 50 mM Tris-HCl, pH 7.5, and 5% (v/v) .beta.-mercaptoethanol. To the mixture was further added and mixed cesium chloride to the final concentration of 1 g/1.1 ml. The solution (5.3 ml) was gently overlayed on a 5.7M cesium chloride solution (3.5 ml) in a centrifuge tube, and centrifuged at 30,000 rpm for 12.5 hours using Hitachi RPS40T Rotary, which gave RNAs as a precipitate. The precipitate was dissolved in a solution (400 .mu.l) comprising 0.5% sodium lauryl sulfate, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.5, followed by phenol-chloroform extraction and ethanol precipitation. The resultant RNAs (about 60 .mu.g) was dissolved in a solution (40 .mu.l) comprising 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA. A 2[illegible] .mu.l aliquot of the solution provided about 2.4 .mu.g of mRNA containing poly(A) by means of mRNA PURIFICATION KIT (Pharmacia). The poly(A)-containing mRNA (1.1 .mu.g) was dissolved in water (10 .mu.l). To the solution were added oligo d(T) 12-18 primer (1.5 .mu.g) (Pharmacia), 1 mM 4 dNTP (3 .mu.l) (Takara Shuzo), reverse transcriptase (40 U) (Life Science), RNase inhibitor (20 U) (Takara Shuzo), 5.times.concentrated transcriptase buffer (7 .mu.l) comprising 250 mM Tris-HCl, pH 8.3, 40 mM magnesium chloride, and 300 mM potassium chloride, and additionally water to make a total volume of 35 .mu.l. The mixture was allowed to react at 41.degree. C. for one hour, followed by ethanol precipitation to obtain cDNA.

59 The cDNA thus obtained was dissolved in water (15.5 .mu.l). To the solution were added a 5.times.terminal deoxynucleotide transferase buffer (4.8 .mu.l) (25 mM Tris-HCl, pH 7.5, 50 mM magnesium chloride), terminal deoxynucleotide transferase (12 U) (Pharmacia), and 10 mM dCTP (3.4 .mu.l) (Takara Shuzo) to make a total volume of 24 .mu.l, and the mixture was allowed to react at 37.degree. C. for 1.5 hours to add poly C to 3' terminal of cDNA. After completion of the reaction, the enzyme were inactivated by heating at 70.degree. C. for 10 minutes.

60 PCR was conducted based on the cDNA thus obtained as a template using Perkin Elmer Cetus DNA THERMAL Cycler following the manual provided by the manufacturer. Thus, to the above reaction mixture (5 .mu.l) were added, as a primer for amplifying cDNA encoding variable region of the heavy chain, poly G (18 nucleotides) which hybridizes at tail added to 3' terminal of the cDNA (40 pmol), a single stranded DNA primer (5' nucleotides, corresponding to the region spanning from part of the variable region (113-119 amino acid sequence in Sequence Listing No. 5) to the constant region which is common to all human IgG (25 pmol (Nucleic Acids Research 14 1779, 1986), poly G as a primer for amplifying cDNA encoding variable region of the light chain (40 pmol), a single stranded DNA primer (21 nucleotides, corresponding to the region spanning from J region of human .kappa. chain (113-114 amino acid sequence of Sequence Listing No. 6) to the constant region (The Journal of Biological Chemistry 257 1516, 1982; Cell 22 197, 1981) (40 pmol), 10.times.PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM potassium chloride, 15 mM magnesium chloride, 0.1% (w/v) gelatin (10 .mu.l), 10 mM 4 dNTP (2 .mu.l) (Takara Shuzo), and Taq DNA polymerase (2.5 U) (Takara Shuzo)), and further water to make a final volume of 100 .mu.l. Thirty cycles of incubations at 94.degree. C. for one minute (denaturing step) at 55.degree. C. for two minutes (annealing step) and at 72.degree. C. for

three minutes (elongation step) were conducted and further incubation at 72.degree. C. for seven minutes was added. Reaction mixture was subjected to ethanol precipitation, and resultant precipitates were dissolved in water (50 .mu.l).

61 To the aqueous solution were added Klenow fragment (2 U) (Takara Shuzo), 1 mM 4 dNTP (4 .mu.l), and 10.times.blunting buffer (500 mM Tris-HCl, pH 7.6, 100 mM magnesium chloride) (4 .mu.l), 40 .mu.l in total, and the mixture was allowed to react at 37.degree. C. for 30 minutes to obtain a double-stranded cDNA having blunt ends.

62 b. Determination of base sequence of cDNA

63 The cDNA solution obtained above was subjected to 2% agarose electrophoresis, and a band was observed at about 500 bp. The band was cut away from the agarose gel. The cDNA was inserted into a cloning vector pUC118 at SmaI site, and the base sequence was determined by dideoxy method, which revealed that among total base sequence of the PCR fragment, the base sequences encoding variable regions of the heavy and light chains were respectively those shown in Sequence Listing Nos. 5 and 6.

64 The amino acid sequences of variable regions of heavy and light chains of antibody GAH produced by the above-mentioned hybridoma were deduced from the base sequences determined above and are respectively shown in Sequence Listing Nos. 7 and 8. Based on the base sequences determined, antibody GAH was shown to belong to IgG1 subclass. The DNA fragment, the base sequence of which has been determined, can be prepared by means of DNA synthesizer with good reproducibility, and therefore, the acquisition of the DNA fragment does not require the repetition of the above procedure.

65 EXAMPLE 4

66 Establishment of Human Monoclonal Antibody 1-5-1 Producing Hybridoma by Cell Fusion between Lymphocyte Derived from Cancer Associated Lymph Node and Mouse Myeloma

67 (1) Preparation of Lymphocyte

68 In substantial accordance with the procedure described in Example 1 (1), lymphocytes (3.times.10.sup.7) were prepared starting from cancer associated lymph node extracted from a patient with lung cancer.

69 (2) Cell Fusion

70 Lymphocyte cells obtained above were fused with mouse myeloma cells (3.times.10.sup.6) using polyethyleneglycol (Boehringer-Mannheim) according to the conventional method. In the same manner as Example 1 (3), the fused cells were suspended in HAT addition medium to obtain cell density of 7.5.times.10.sup.5 /ml and plated on a 96 well plate at a ratio of 100 .mu.l/plate. Half of the culture medium was substituted with HAT addition medium from time to time and the culture was continued until hybridoma's colonies appeared. The hybridoma's colonies were observed in all of the wells. In the same manner as in Example 1 (4), the supernatant of the culture in each well was tested on the reactivity to fixed cancer cell lines such as colon cancer cell line C-1 and stomach cancer cell line MKN45, in accordance with the procedure described in Experiment 1 (2)-a. Positive wells were 16.3% (94 well) against C-1 and 6.3% (36 wells) against MKN45, and 4 wells showed positive reaction to both lines.

71 Cloning of hybridoma cells was conducted using the wells which showed positive reaction to both lines. The cloning was conducted three times by means of limiting dilution method, and hybridoma clone 1-5-1 was established.

72 EXAMPLE 5

73 Purification and Labeling of Monoclonal Antibody 1-3-1

74 (1) Culture of Hybridoma 1-3-1 and Purification of Monoclonal Antibody 1-3-1

75 For culture of hybridoma 1-3-1, eRDF culture medium (Gokuto Seiyaku) to which 3% of the serum described in Example 2 (1) had been added was used. The culture of hybridoma 1-3-1 was then charged into a Protein A-agarose column, and adsorbed antibody was then eluted out to obtain purified antibody 1-3-1. The antibody was confirmed to be a pure IgM by SDS-PAGE.

76 (2) Fluorescent Labeling of Antibody 1-3-1

77 The purified antibody 1-3-1 was labeled by FITC according to the method described in Example 2 (2). Absorbance of fractions containing labeled antibody was measured at OD.sub.280 nm and OD.sub.495 nm, and labeling degree was determined. F/P ratio was 6.1.

78 EXPERIMENT 4

79 Study on Reactivity of Human Monoclonal Antibody to Cancer Cell Lines

80 (1) Cancer Cell Lines and Preservation thereof

81 Human colon cancer cell line C-1 and stomach cancer cell line MKN45 were preserved and grown at 37.degree. C. and 5% CO.sub.2 conditions in Culture Medium B in the same manner as described in Experiment 1 (1).

82 (2) Study on Reactivity to Living Cancer Cell Lines

83 Cancer cells were cultured in a flask or Petri dish and culture supernatant was discarded. To the residue was added PBS solution containing 1.2% EDTA, and the mixture was left to stand at room temperature for 30 minutes allowing the cells to float. The cells were washed with Culture Medium B by centrifugation and suspended in PBS so as to make the cell density of about 1.times.10.sup.6 /200 .mu.l. Antibody 1-3-1 obtained in Example 5 (1) was added to the above solution to make the final concentration of the antibody of 50 .mu.g/ml, and the mixture was allowed to react at 0.degree. C. for 60 minutes. The suspension was centrifuged at 2000 rpm for 5 minutes and the supernatant was discarded. To the remaining cells was added FITC labeled anti-human antibody solution (200 .mu.l) (Capel diluted with 1% BSA-containing PBS by 500 times, and the resulting cell suspension was kept at 0.degree. C. for 60 minutes. The suspension was centrifuged at 2000 rpm for 5 minutes to remove the supernatant, and the remaining cells was suspended in and washed with PBS (1 ml) by centrifugation, and the cells were finally suspended in PBS (300 .mu.l) containing PI (10 .mu.g/ml). The resultant cell suspension was subjected to FCM, and magnitude of fluorescence (FITC and PI) bonded to particular cell was determined. The reactivities of antibody 1-3-1 to colon cancer cell line C-1 and stomach cancer cell line MKN45 are respectively shown in FIGS. 3 and 4 of the accompanying drawings. In the figures, the abscissa shows fluorescence intensity per cancer cell and the ordinate shows the number of the cancer cells. As a control, a commercially available IgM antibody (Capel) was used, and the reactivities of the IgM antibody to the above-identified cancer cells were determined. In the figures, the dotted line and solid line show the reactivities of antibody 1-3-1 and the control respectively. These figures show that antibody 1-3-1 has a strong binding ability to cancer cells.

84 EXPERIMENT 5

85 Reactivity of Human Monoclonal Antibody 1-3-1 to Cells Derived from Fresh Cancer Tissue and Non-Cancer Tissue

86 In order to study a binding specificity of antibody 1-3-1 to cancer cells, normal cells were simultaneously isolated from fresh tissue belonging to the same organ of the same patient, from which cancer cells were obtained, and reactivities of antibody 1-3-1 to respective cells were measured. Isolation of cells from the tissue was conducted according to Iwata's method as described in Experiment 3.

87 The reactivity to the cells obtained above was determined by FCM in the same manner as previously described in the living cancer cells. However, the cells were washed with Culture Medium B, suspended in serum derived from healthy volunteers, which serum contained fluorescent labeled antibody 1-3-1 (final concentration of 50 .mu.g/ml) prepared in Example 5 (2), to the cell density of about 1.times.10.sup.6 /200 .mu.l. The suspension was allowed to react at 0.degree. C. for 60 minutes and subjected to centrifugation at 2000 rpm for 2 minutes to remove the supernatant. The remaining cells were suspended in PBS (1 ml) and washed by centrifugation. The cells were resuspended in PBS (500 .mu.l) containing PI (10 .mu.g/ml), and the suspension was subjected to FCM. The amount of fluorescent (FITC and PI) bonded to a particular cell was measured. Markers (3 species) for determining the amount of fluorescence (quantitative kit as previously prescribed) were subjected to FCM under the same condition. Average amount of FITC bonded to a single cell was calculated. Based on the average amount and F/P ratio of labeled antibody calculated in Example 5 (2), the average number of antibodies bonded to a living cancer cell was calculated. The results are shown in Table 4.

TABLE 4

	Colon		Stomach	
Antibody	Cancer Cells	Non-cancer Cells	Cancer Cells	Non-cancer Cells
1-3-1	1.5 .times. 10.sup.4			
		1.4 .times. 10.sup.4		
			1.3 .times. 10.sup.3	
				5.0 .times. 10.sup.3
Control	0.15 .times. 10.sup.4			
		0.94 .times. 10.sup.4		
			9.2 .times. 10.sup.3	
				8.5 .times. 10.sup.3

88 The reactivity of the human monoclonal antibody 1-3-1 to non-cancer cells was the same level as, or less than, that of the antibody which was derived from peripheral blood of healthy volunteers and fluorescent-labeled in the same manner as antibody 1-3-1, while the average number of antibodies bonded to cancer cells is remarkably higher than that in the non-cancer cells. In addition, the number of antibodies bonded to cancer cells was 11 times greater than that in the control antibody both in stomach and colon cancer. These results indicate that antibody 1-3-1 recognizes an antigen dominantly expressed on the surface of cell membrane of cancer cells.

89 EXAMPLE 6

90 (1) Determination of Subclass of Light Chain of Monoclonal Antibody 1-3-1

91 In order to determine the subclass of the light chain of antibody 1-3-1, the same procedure as described in Example 3 was repeated except that antibody 1-3-1 obtained in Example 5 (1) was used in place of antibody 3AH. The light chain of antibody 1-3-1 reacted with anti-human .lambda. chain antibody, which was detected through the appearance of coloured band. This revealed that the

light chain was .lambda. chain.

92 (3) Preparation of Gene Encoding Monoclonal Antibody 1-3-1 and Determination of Base Sequence

93 a. Preparation of cDNA encoding antibody 1-3-1 by means of PCR

94 According to the method detailed below, poly(A) containing RNAs were prepared from antibody 1-3-1 producing hybridoma obtained in Example 4 (2) using guanidine thiocyanate-lithium chloride method (DNA 7, 339, 1988).

95 In the same manner as described in Example 3 except that the number of hybridoma cells used was 2.times.10.sup.8, the mRNA was prepared. The resultant RNAs (about 1.5 mg) was dissolved in a solution (1 ml) comprising 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA. A 250 .mu.l aliquot of the solution provided about 10 .mu.g of mRNA containing poly(A) after purification by means of mRNA PURIFICATION KIT (Pharmacia). Following the procedure described in Example 5, the poly(A)-containing mRNA (4.5 .mu.g) was dissolved in water (10 .mu.l), and to the solution were added oligo(dT)$_{12-18}$ primer (0.5 .mu.g), 10 mM 4 dNTP (2 .mu.l), reverse transcriptase (40 U), RNase inhibitor (30 U), 5.times.reverse transcriptase buffer (4 .mu.l), and additionally water to make a total volume of 20 .mu.l. The mixture was allowed to react at 42.degree. C. for one hour, followed by ethanol precipitation to obtain cDNA.

96 The cDNA thus obtained was dissolved in water (20 .mu.l). To the solution were added a 5.times.terminal deoxynucleotide transferase buffer (5 .mu.l), terminal deoxynucleotide transferase (11 U), and 10 mM dGTP (2.5 .mu.l) to make a total volume of 45 .mu.l by adding water (6.5 .mu.l), and the mixture was allowed to react at 37.degree. C. for 1 hour to add poly d(G) at 3' terminal of cDNA. After completion of the reaction, the enzymes were inactivated by heating at 65.degree. C. for 10 minutes.

97 PCR was conducted using the cDNA thus obtained as a template. That is, to the above reaction mixture (2.5 .mu.l) were added, as a primer for amplifying cDNA encoding variable region of the heavy chain, poly C (14 nucleotides) which hybridizes dG tail added to 3' terminal of the cDNA (25 pmol), a single stranded DNA primer (17 nucleotides) corresponding to the base sequence of constant region of IgM shown in Sequence Listing No. 7 (0.5 pmol.) (Nucleic Acids Research 9, 4213, 1981), poly G as a primer for amplifying cDNA encoding variable region of the light chain (25 pmol), a single stranded DNA primer (19 nucleotides) (25 pmol) corresponding to the base sequence of constant region of .lambda. chain, shown in Sequence Listing No. 8 (Nature 294 536, 1981). The mixture was treated in the same manner as described in Example 3, which provided a double-stranded cDNA having blunt ends.

98 b. Determination of base sequence of cDNA

99 The cDNA solution obtained above was subjected to 1% agarose electrophoresis, and a band was observed at about 1.7 kbp. The band was cut away from the agarose gel. The cDNA was inserted into a cloning vector pUC119 at SmaI site, and the base sequence was determined by dideoxy method, which revealed that among total base sequence of the PCR fragment, the base sequence encoding variable regions of the heavy and light chains were respectively those shown in Sequence Listing Nos. 9 and 10.

100 The amino acid sequences of variable regions of heavy and light chains of antibody 1-3-1 produced by the above-mentioned hybridoma were deduced from the base sequences determined above and are respectively shown in Sequence Listing Nos. 11 and 12. The DNA fragment, the base sequence of which has been determined, can be prepared by means of DNA synthesizer with good reproducibility, and therefore, the acquisition of the DNA fragment does not require the repetition of the above procedure.

101 EXAMPLE 7

102 Preparation of Adriamycin-Containing Liposome Bonded to Antibody GAH

103 a. Preparation of Thiolated Antibody

104 Anti-cancer antibody GAH (IgG) was dissolved in 0.1M--acetate buffer (pH 4.0), and pepsin (1/40 mol) (Cooper Biomedical) was added thereto. The mixture was allowed to react overnight to prepare F(ab').sub.2. Chromatography over cation-exchange resin (mono S) (Pharmacia) isolated F(ab').sub.2. The solvent used was a linear gradient of 0.1M--acetate buffer (pH 4.3) containing 0-0.3M NaCl. To the isolated F(ab').sub.2 in 0.1M--acetate buffer (pH 4.5) containing 0.15M NaCl was added DTT at a ratio of 1/2 .mu.l of 10% DTT/1 mg antibody. The mixture was left to stand at room temperature for 30 minutes. After completion of reaction, the mixture was passed through a gel filtration column (PD-10) equilibrated with PBS for desalification to obtain thiolated Fab'.

105 b. Thiolation of polyethylene glycol

106 L-cysteine (4.8 mg) was dissolved in 0.4M borate buffer (10 ml), and 1,4-bis (polyethylene glycol (6-chloro-s-triazine (210mg) (activated PEG) (Seikagaku Kogyo) was added thereto. The mixture was allowed to react at room temperature overnight. To the resultant PEG bonded with cysteine was added DTT (62 mg), and the mixture was allowed to react at 37.degree. C. for 6 hours to obtain a solution containing PEG bonded with cysteine. The solution was gel filtrated (GH-25, Seikagaku Kogyo) for desalting, and the solvent was substituted by 10 mM phosphate buffer (pH 7.4) and 0.15M-NaCl (PBS). The solution was added to thiopropyl Sepharose 6B (Pharmacia) equilibrated with PBS, and non-bonded substances were washed away by PBS. Cysteine-binding PEG adsorbed to the gel was eluted out by PBS containing 50 mM-DTT, which was then subjected to gel filtration to remove excessive DTT. This gave thiolated PEG.

107 c. Maleimidation of dipalmitoylphosphatidylethanolamine

108 Dipalmitoylphosphatidylethanolamine (127 mg), N-(.epsilon.-maleimidocaproyloxy)succinimide (EMCS) (51 mg), and triethylamine were added to a chloroform-methanol (9:1) solution (44 .mu.l), and the mixture was allowed to react for 3 hours under nitrogen gas. Additional EMCS (20 mg) was added and the mixture was allowed to react at room temperature for further 2 hours. After confirmation of negative ninhydrin reaction of the reaction mixture, the mixture was evaporated to dryness under reduced pressure and the residue was dissolved in a trace amount of chloroform. The maleimidated dipalmitoylphosphatidylethanolamine thus obtained was purified by chromatography over UNISIL (Gasukuro Kogyo) equilibrated with chloroform, using a chloroform/methanol (10:1) solution as an eluent.

109 d. Preparation of liposome containing adriamycin bearing maleimide group

110 Polin lipid mixture (100 mg) (Nippon Seika), which consists of dipalmitoylphosphatidylcholine (DPPC), cholesterol (Chol), and maleimidated dipalmitoylphosphatidylethanolamine at a ratio of 10:1:0.3 (mol) was added to 0.3M citrate buffer (pH 4) (1 ml) and admixed. Freezing and thawing of the mixture was repeated 7 times to achieve hydration. This gave multimera liposome. The liposome was charged in an extruder (Lipex.times.Biomembranes) equipped with a polycarbonate membrane (Nuclepore; Microscience) having a pore size of 100 nm and kept at 45.degree. C. Repeated pressure-filtration (11 times) gave a dressed liposome. The liposome solution was neutralized with addition of 1N NaOH solution, and to the neutral solution was added one tenth by weight of adriamycin (Kyowa Hakko) with respect to the lipid components while being kept at 5.degree. C. More than 97% of adriamycin was positively enclosed into the liposome according to the pH slope between the inside and outside of the liposome to give a liposome into which adriamycin bearing maleimide group had been encapsulated.

111 e. Binding of maleimide group-bearing adriamycin-encapsulated liposome to thiolated antibody and PEG modification

112 To the adriamycin-encapsulated liposome obtained above (lipid components: 100 mg) was added thiolated Fab' antibody (5 mg), and the mixture was allowed to react at 37.degree. C. for 5 hours. To the reaction mixture was added thiolated PEG (5 .mu.mol), and the mixture was allowed to react in PBS at room temperature for 6 hours to obtain adriamycin-encapsulated liposome bonded to antibody and modified with PEG. The latter was further subjected to gel filtration using SEPHAROSE CL6B (Pharmacia; "SEPHAROSE" is a registered TM for ion exchange gel) to remove non-reacted cysteine-binding PEG.

113 EXPERIMENT 4

114 Confirmation of Pharmaceutical Effectiveness of Adriamycin-Encapsulated Liposome Bonded to Antibody GAH and Modified with PEG

115 Study on anti-cancer effect of antibody GAH was conducted in the manner as described below using human stomach cancer cell line MKN45 which had shown reactivity to antibody GAH and accumulative behavior in transplantation to nude mouse.

116 Cultured MKN45 cells (1.times.10.sup.6) were subcutaneous-transplanted to nude mouse. Experiment started when the cancer weight became about 100 .mu.g after ten days from the transplantation (FIG. 5). Adriamycin-encapsulated liposome bonded to antibody GAH and modified with PEG was administered to mouse via caudal vein at a dose corresponding to 5 mg/kg of adriamycin. day 0, 3, 7 (shown by mark .diamond. in FIG. 5). As a control, phosphate buffered physiological saline (shown by mark .diamond-solid.), adriamycin (shown by mark .quadrature.), and adriamycin-encapsulated liposome modified with PEG (shown by mark .times.) were administered to mice (each 6-7 animals). Time-course measurement of growth of cancer was conducted by means of Battle-Columbus method wherein presumptive cancer weight was determined according to the formula: (short diameter).times.(short diameter).times.(long diameter)/2, and compared with that determined at the beginning of the experiment.

117 In FIG. 5, the abscissa shows time-lapse (days) after beginning of the experiment, and the mark (downarw.) indicates the administration of the pharmaceutical formulation of the invention. FIG. 5 clearly shows that the formulation of the invention, adriamycin-encapsulated liposome bonded to antibody GAH, possesses potent anti-cancer effect. It is apparent, therefore, that human monoclonal antibodies of the invention allow continuous and long term "targeting therapy" of cancer tissue or organ with the help of anti-cancer agents or toxins.

SEQUENCE LISTING
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(iii) NUMBER OF SEQUENCES: 42
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(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE: human IgG antibody
(A) ORGANISM:

(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
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(iv) ANTI-SENSE:
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(F) TISSUE TYPE:
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(C) IDENTIFICATION METHOD:

(ix) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
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(D) TOPOLOGY: linear
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(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
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(B) STRAIN:
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(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human
antibody MAB
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(B) CLONE:
(viii) POSITION IN GENOME:
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(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
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(B) TITLE:
(C) JOURNAL:
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(I) FILING DATE:
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(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(F) STRAIN:
(G) INDIVIDUAL ISOLATE:
(H) DEVELOPMENTAL STAGE:
(I) HAPLOTYPE:
(J) TISSUE TYPE:
(K) CELL TYPE: Hybridoma producing human
antibody
(L) LIBRARY:
(M) CLONE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
GACATCCTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTG45
GGCGAGAGGGCCACCATCAACTGCAAGTCCAGCCAGAGTGTTTTA90
TACAACTCCAACAATAAGAAAATACTTAGCTTGGTACCAGCAGAAA135
CCAGGACAGCCTCCTAAGTTGCTCATTTACTGGGCATCTACCCGG180
GAATCCGGGGTCCCTGACCGATTCAGTGGCAGCGGGTCTGGGACA225
GATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCA270
GTTTATTACTGTCAGCAATATTATAGTACTCCGTGGACGTTCGGC315
CAAGGGACCAAGGTGGAAATCAAACGA342
(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human
antibody MAB
(H) CELL LINE:
(I) CLONE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) MAP POSITION:
(B) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
GlnValGlnLeuGlnGlnSerGlyProGlyLeuValLysProSer

1 5 10 15
GlnThrLeuSerLeuThrCysThrValSerGlyGlySerIleSer
20 25 30
SerCysGlyPheTyrTrpAsnTrpIleArgGlnHisProGlyLys
35 40 45
GlyLeuGluTrpIleGlyTyrIleTyrTyrSerGlySerThrTyr
50 55 60
TyrAsnProSerLeuLysSerArgValThrIleSerLeuAspThr
65 70 75 80
SerLysAsnGlnPheSerLeuLysLeuSerSerLeuThrAlaAla
85 90 95
AspThrAlaValTyrTyrCysAlaArgSerThrArgLeuArgGly
100 105 110
AlaAspTyrTrpGlyGlnGlyThrMetValThrValSerSer
115 120
(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human
antibody Ab
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
AspIleValLeuThrGlnSerProAspSerLeuAlaValSerLeu
1 5 10
GlyGluArgAlaThrIleAsnCysLysSerSerGlnSerValLeu
20 25
TyrAsnSerAsnAsnLysLysTyrLeuAlaTrpTyrGlnGlnLys
35 40 45
ProGlyGlnProProLysLeuLeuIleTyrTrpAlaSerThrArg
50 55 60
GluSerGlyValProAspArgPheSerGlySerGlySerGlyThr
65 70
AspPheThrLeuThrIleSerSerLeuGlnAlaGluAspValAla
85 90
ValTyrTyrCysGlnGlnTyrTyrSerThrProTrpThrPheGly
95 100 105
GlnGlyThrLysValGluIleLysArg
110
(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE: human IgM antibody
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
CGAGGSSGWWAGGGTTI7
(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE: human IgM antibody
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GAAGTCTCCAGAAAGGGT

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3** base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
CAGCTGCAGGTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCG4

GAGACCCTGTCCCTCACCTGCACTGTCTCTGGTGGCTCCATCAGC90
AGTAGTAGTTACTACTGGGGCTGGATCCGCCAGCCCCCAGGGAAG135
GGGCTGGAGTGGATTGGGAGTATCTATTATAGTGGGAGCACCTAC180
TACAACCCGTCCCTCAAGAGTCGAGTCACCATATCCGTAGACACG225
TCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCA270
GACACGGCTGTGTATTACTGTGCGAGGGGGAGCTACGGGGGCTAC315
TACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTC360
TCCTCA366
(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 324 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:

(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
TATGAGCTGACAGCCACCCCTCGGTGTCAGTGTCCCCAGGACAG45
ACGGCCAGGATCACCTGCTCTGGAGATGCATTGCCAAAGCAATAT90
GCTTATTGGTACCAGCAGAAGCCAGGCCAGGCCCCTGTGCTGGTG135
ATAATAAAAGACAGTGAGAGGCCCTCAGGGATCCCTGAGCGATTC180
TCTGGCTCCAGCTCAGGGACAACACAGTCACGTTGACCATCAGTGGA225
GTCCAGGCAGAAGACGAGGCTGACTATTACTGTCAATCAGCAGAC270
AGCAGTGGTACTTATGAGGTATTCGGCGGAGGGACCAAGCTGACC315

GTCGTAGGT324
(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 142 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
GlnLeuGlnLeuGlnGluSerGlyProGlyLeuValLysProSer
1 5 10 15
GluThrLeuSerLeuThrCysThrValSerGlyGlySerIleSer
20 25 30
SerSerSerTyrTyrTrpGlyTrpIleArgGlnProProGlyLys
35 40 45
GlyLeuGluTrpIleGlySerIleTyrTyrSerGlySerThrTyr
50 55 60
TyrAsnProSerLeuLysSerArgValThrIleSerValAspThr
65 70 75 80
SerLysAsnGlnPheSerLeuLysLeuSerSerValThrAlaAla
85 90 95
AspThrAlaValTyrTyrCysAlaArgGlySerTyrGlyGlyTyr
100 105 110
PheTyrTyrGlyMetAspValTrpGlyGlnGlyThrThrValThrVal
115 120 125
SerSer
(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 138 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
TyrGlyAsnThrGlnProProSerValSerValSerProGlyGln
1 5 10 15
ThrAlaArgIleThrCysSerGlyAspAlaLeuProLysGlnTyr
20 25 30
AlaTyrTrpTyrGlnGlnLysProGlyGlnAlaProValLeuVal
35 40 45
IleTyrLysAspSerGluArgProSerGlyIleProGluArgPhe
50 55 60
SerGlySerSerSerGlyThrThrValThrLeuThrIleSerGly
65 70 75
ValGlnAlaGluAspGluAlaAspTyrTyrCysGlnSerAlaAsp
80 85 90
SerSerGlyThrTyrGlyValPheGlyGlyGlyThrLysLeuThr
95 100 105
ValLeuGly
(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: hybridoma producing human monoclonal
antibody, an antigen to which exists on the surface of
cancer cell membrane
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION: 4
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: /note ="Cys or Ser"
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION: 5
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: /note ="Gly or Ser"
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION: 6
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: /note ="Phe or Tyr"
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
IleSerSerXaaXaaXaaTyrTrp
15
(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:

(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: hybridoma producing human monoclonal antibody, an antigen to which exists on the surface of cancer cell membrane
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION: 5
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: note ="Tyr or Ser"
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
IleGlyXaaIleTyrTyrSerGlySerThrTyrTyr
1 5 10
(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: hybridoma producing human monoclonal antibody, an antigen to which exists on the surface of cancer cell membrane
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:

(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION: 1
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: /note ="Ala or Met"
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION: 4
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: /note ="Tyr or Val"
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
GlyXaaAspXaa
(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody GAH
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:

(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
IleSer Tyr Asp GlyPhe TyrTrp Asn
1 5
(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody GAH
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
IleGlyTyrIleTyrTyrSerGlySerThrTyrTyr
1 5 10
(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody GAH
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) UNIT MAP SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
SerTyrAlaMetAsnGlyAlaAspTyr
1 5
(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody GAH
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
LysSerCysAspLysSerValLeuTyrAsnSerAsnAsnLysLysTyrLeu
1 5 10 15
Ala

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody GAH
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:

(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
TrpAlaAsnThrAsnAspGlnSer
1 5
(2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody GAH
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
GlnGlyGlyGlyGlyThrProTrpThr
1 5
(2) INFORMATION FOR SEQ ID NO:22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
IleSerCysArgAlaSerGlnTyrIleTyrTrpGlyTrp
1 5 10
(2) INFORMATION FOR SEQ ID NO:23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:

(A) LIBRARY:
(B) CLONE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
IleGlyGlyIleTyrTyrSerGlySerThrTyrTyrAsnPro
1510
(2) INFORMATION FOR SEQ ID NO:24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:

(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
GlySerTyrGlyGlyTyrTyrTyrGlyMetAspVal
1510
(2) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
AspAlaLeuArgGlyGlnTyrAlaTyr
15
(2) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
LysAspGlySer
]
(2) INFORMATION FOR SEQ ID NO:27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
GlnSerAlaAspSerSerGlyThrTyrGluVal
1 10
(2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human monoclonal
antibody, an antigen to which exists on the surface of
cancer cell membrane
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:

(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
ATCA[illegible]AGTWGTR[illegible]TWTTACTGG24
(2) INFORMATION FOR SEQ ID NO:29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human monoclonal antibody, an antigen to which exists on the surface of cancer cell membrane
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
A[illegible]TG[illegible]TATTAYAGTGGGAGCACCTACTAC36
(2) INFORMATION FOR SEQ ID NO:30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1[illegible] base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human monoclonal antibody, an antigen to which exists on the surface of cancer cell membrane
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(vii) POSITION IN GENOME:
(A) CHROMOSOME SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
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(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
GGKRTGGACEWCCT
(2) INFORMATION FOR SEQ ID NO:31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody GAH
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(vii) POSITION IN GENOME:
(A) CHROMOSOME SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
ATCAGTAGTTGTGGTTTCTACTGG24
(2) INFORMATION FOR SEQ ID NO:32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody GAH
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:

(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
ATTGGGTAAACTATTACAGTGGGAGCACCTACTAC36
(2) INFORMATION FOR SEQ ID NO:33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Hybridoma producing human antibody GAH
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:
TGTACTGGACTTACGGGGGGCTGACTAC27
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(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:

(D) DEVELOPMENTAL STAGE:
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(H) CELL TYPE: Hybridoma producing human antibody GAH
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(B) TITLE:
(C) JOURNAL:
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(H) DOCUMENT NUMBER:
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(H) CELL LINE:
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(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
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(H) CELL LINE:

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L2: Entry 185 of 185 File: USPT Nov 23, 1993

US-PAT-NO: 5264221
DOCUMENT-IDENTIFIER: US 5264221 A

TITLE: Drug-containing protein-bonded liposome

DATE-ISSUED: November 23, 1993

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INT-CL: [05] A61K 9/127

US-CL-ISSUED: 424/450; 428/402.2, 436/829, 935/54, 530/812
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FIELD-OF-SEARCH: 424/450, 424/417, 436/829, 428/402.2, 264/4.1, 530/402, 530/403, 530/810, 530/812, 935/54

PRIOR-ART-DISCLOSED:

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Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4429008	January 1984	Martin	424/450
☐	5059421	October 1991	Loughrey et al.	424/418

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FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
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World Patent Index Latest, No. 89-335876.

ART-UNIT: 152

PRIMARY-EXAMINER: Page; Thurman K.

ASSISTANT-EXAMINER: Kishore; G. S.

ABSTRACT:

A drug-containing protein-bonded liposome comprising a liposome containing a drug and having maleimide residues on its surface, and a protein and residues of a compound having a polyalkylene glycol moiety, bonded via respective thiol groups to the maleimide residues.

15 Claims, 2 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 2

BRIEF SUMMARY:

1 The present invention relates to selective chemotherapeutic drugs for various diseases including cancer. More particularly, it relates to a drug-containing protein-bonded liposome.

2 A missile therapeutic agent whereby a drug can be concentrated at a required active site utilizing a specific reactivity of an antibody, is expected to be useful in various medical fields including a field of cancer treatment in view of its high level of effectiveness and low side effects. For realization of such a missile therapeutic agent, it is important to establish a technology for

combining an antibody and a drug. Heretofore, it has been attempted to bond an antibody and a drug by a method wherein a drug is chemically modified for bonding i.e. a method wherein an antibody and a drug are directly bonded, or a method wherein they are bonded via a water-soluble polymer such as dextran. However, with these methods, problems have been pointed out that the amount of a drug which can be bonded per molecule of an antibody is small, and the activities tend to be reduced by the modification of a drug. On the other hand, as a means for transporting a drug in a large amount without modifying the drug, a method has been proposed wherein a drug is contained in a liposome and an antibody is bonded to the surface of the liposome. Namely, an antibody-bonded liposome has been proposed.

3 Also in the field of cancer treatment, anti-cancer drug-containing antibody-bonded liposomes have been prepared, and many research institutes have reported excellent antitumor effects thereof (Kono et al., Cancer Res. 47, 4471 (1987), Hashimoto et al., Japanese Unexamined Patent Publication No. 191124/1988). However, at the same time, some problems of antibody-bonded liposomes have been pointed out. Namely, many of antibody-bonded liposomes administered are likely to be captured by organs of reticulo-endothelial system such as liver and spleen, whereby no adequate effects tend to be obtained (Hashimoto et al., Cancer Res. 47 3515 (1988)).

4 On the other hand, it has been proposed to bond e.g. polyethylene glycol to a liposome as a method of improving the general properties of a liposome, such as leakage of the contained substance, agglomeration and a nature of being captured by organs of reticuloendothelial system (Japanese Unexamined Patent Publications No. 248717/1989 and No. 119613/1990, Alexander D. Bangham et al, FEBS letters 308 2ch (1990)).

5 However, in these methods, lipophilic derivatives of polyethylene glycol and a compound such as a long chain aliphatic acid are mixed with other liposome-constituting lipids, and a polyethylene glycol layer is formed on the liposome surface during the preparation of the liposome, or polyethylene glycol derivatives reactive with amino groups are attached to amino groups introduced to the liposome surface. When such methods are applied to an antibody bonded liposome, bonding of the antibody is likely to be hindered by the polyethylene glycol layer already formed on its surface, or deactivation of the antibody is likely to result. Therefore, the conventional method of incorporating polyethylene glycol has not been primarily intended for application to an antibody-bonded liposome.

6 The present inventors have conducted extensive studies to present a drug-containing antibody-bonded liposome having the nature of being captured in the reticuloendothelial system improved, and as a result, have found it possible to accomplish this object by firstly reacting a protein to which a thiol group is imparted (a thiol modified protein) to a liposome having maleimide groups and then reacting a compound having a moiety of a polyalkylene glycol to which a thiol group is imparted (a thiol-modified polyalkylene glycol) to the remaining maleimide groups.

7 Thus, the present invention provides a drug-containing protein-bonded liposome comprising a liposome containing a drug and having maleimide residues on its surface, and a protein and residues of a compound having a polyalkylene glycol moiety, bonded via respective thiol groups to the maleimide residues.

DRAWING DESCRIPTION:

In the accompanying drawings:

FIG. 1 shows the reactivities of antibody-bonded liposomes to human gastric cancer cell line MKN 45, with respect to polyethylene glycol (PEG)-modified and non-modified liposomes.

The ordinate represents the amount bonded to MKN 45 in terms of the amount of 6-carboxyfluorescein (CF). In this Figure, lip represents the CF-loaded liposome, and antibody-bonded lip represents the CF-loaded antibody-bonded liposome.

FIG. 3 shows the antitumor activities of the adriamycin-containing antibody-bonded PEG-modified liposome against cancer transplanted to nude mouse. The abscissa represents the number of days after initiation of the therapeutic test, and the ordinate represents the assumed tumor weight. In this Figure, represents the day on which the drug was administered; PBS represents a phosphate buffer physiological saline; ADR represents adriamycin alone; PM represents the adriamycin-containing PEG-modified liposome, and PM(GAH) represents the adriamycin-containing human monoclonal antibody-bonded PEG-modified liposome.

DETAILED DESCRIPTION:

1 Now, the present invention will be described in detail with reference to the preferred embodiments.

2 (1) Liposome

3 1 The liposome is composed essentially of phosphatidyl choline, cholesterol and maleimide-modified phosphatidyl ethanolamine. However, a phosphatidic acid such as dipalmitoylphosphatidic acid (DPPA) or the like may be incorporated as a substance imparting an electric charge.

4 As a preferred liposome, a liposome composed of dipalmitoylphosphatidyl choline (DPPC), cholesterol (Chol) and maleimide-modified dipalmitoylphosphatidyl ethanolamine (maleimide-modified DPPE), may be mentioned.

5 2 The maleimide-modified phosphatidyl ethanolamine can be obtained by the reaction of a maleimide-containing compound reactive with an amino group, with an amino group of phosphatidyl ethanolamine (PE). The maleimide-containing compound may be N-(.epsilon.-maleimidocaproyloxy)succinimide, N-succinimidyl 4-(p-maleimidophenyl)butyrate, N-succinimidyl 4-(p-maleimidophenyl)propionate or N-(.gamma.-maleimidobutyryloxy)succinimide. PE may be dipalmitoylphosphatidyl ethanolamine.

6 3 The respective components are used in such proportions that per mol of the phosphatidyl choline, cholesterol is used in an amount of from 0.3 to 1 mol, preferably from 0.4 to 0.6 mol, the maleimide-modified phosphatidyl ethanolamine is used in an amount of from 0.01 to 0.2 mol, preferably from 0.02 to 0.1 mol, and the phosphatidic acid is used in an amount of from 0 to 0.4 mol, preferably from 0 to 0.1 mol.

7 4 For the preparation of the liposome, a conventional method can be used. For example, a lipid mixture having a solvent removed, is hydrated and emulsified by a homogenizer, followed by freezing-thawing to obtain a multilamella liposome. To further adjust it to a proper particle size, it may be subjected to supersonic treatment, high speed homogenizing or press-filtration by a membrane having uniform pores (Hope M. J. et al., Biochimica et Biophysica Acta 812, 55 (1985)).

8 A preferred size of the liposome is not larger than 300 nm, more preferably from 50 to 200 nm.

9 (2 Drug

10 1 As the drug, an antitumor drug such as adriamycin, daunomycin, mitomycin, cisplatin, vincristine, epirubicin, methotrexate, 5FU or aclacinomycin, an aminoglucoside such as gentamicin, a .beta.-lactam antibiotic such as sulpenisillin, a toxin such as ricin A or diphtheria toxin, antisense RNA

against HIV or ras gene, or actinoplanone (Polycyclic xanthones produced from actinoplanone B-3l4 (K. Kobayashi et al., J. Antibiotics 41, 741 (1988)), may be employed.

11) Loading of the drug into the liposome can be conducted by hydrating the lipid with an aqueous drug solution in the case of a water-soluble drug, or by mixing the drug and the lipid in a volatile organic solvent, followed by distilling the solvent off and hydrating the mixture of the drug and the lipid to embed the drug in the liposome, in the case of a fat-soluble drug. Further, in the case of adriamycin, daunomycin or epirubicin, loading can be conducted by a remote loading method utilizing a pH gradient (Lawrence D. Mayer et al., Cancer Res. 49, 5922 (1989)).

12 (3) Thiol-Modified Protein

13) As the protein bonded to the liposome, various physiologically active substances including an antibody, EGF and BGF, may be employed. Preferred is an antibody. The antibody is an antibody reactive with the virus, bacteria, cells or tissue to be treated. For example, polyclonal antibodies of various animals, a mouse monoclonal antibody, a human-mouse chimeric antibody and a human monoclonal antibody may be employed. Among them, a human monoclonal antibody is preferred in the sense that it is not a protein of a foreign animal.

14) Introduction of thiol groups to the protein can be conducted by a method wherein a compound is employed which is commonly used for thiol-modification of a protein and which is reactive with an amino group of the protein, such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (Carlsson, J. et al., Biochem. J. 173, 723 (1978)) or iminothiolane, mercaptobutyrimidate (Traut, R. R. et al., Biochemistry 12, 3266 (1973)), or the like. In the case of an antibody, a method may be employed wherein endogenous disulfide groups are reduced to thiol groups. For bonding an antibody and a liposome, the latter method utilizing endogenous thiol groups is preferred from the viewpoint of the maintenance of the activities. When IgG is employed, it is subjected to F(ab')2 modification with an enzyme such as pepsin, followed by reduction with e.g. dithiothreitol to obtain Fab', whereupon thiol groups formed in Fab' are subjected to the bonding reaction with the liposome (Martin, F. J. et al., Biochemistry, 20, 4229 (1981)). In the case of IgM, H-chain is reduced under a mild condition in accordance with a method of Miller et al. (J. Biol. Chem. 257, 286 (1982)), whereupon thiol groups of Fc moiety of IgMs thereby obtained, are subjected to the bonding reaction with the liposome.

15) Bonding of the maleimide group-containing liposome and the thiol-modified protein can be accomplished by reacting them in a neutral buffer solution (pH 6.5 to 7.5) for from 1 to 15 hours.

16 (4) Compound Containing a Thiol-Modified Polyalkylene Glycol Moiety

17) As the polyalkylene glycol moiety of the compound, polyethylene glycol or polypropylene glycol may, for example, be mentioned. Preferred is polyethylene glycol, and its degree of polymerization is preferably from 2 to 400.

18) To introduce thiol groups to polyalkylene glycols, various thiol-modification reactions which are commonly used for hydroxyl groups, amino groups, carboxyl groups and triazine, may be employed. Specific examples will be given below with respect to the case of polyethylene glycol, but it should be understood that the present invention is by no means restricted by such specific examples.

19 Namely, there are a method wherein monomethoxypolyoxy ethyleneamine and various thiolcarboxylic acids are dehydrated and condensed, a method wherein pyridyl dithiopropionyl group is introduced into monomethoxypolyoxy ethyleneamine by SPDP, followed by reduction, a method wherein thiol is introduced into monomethoxypolyoxy ethyleneamine by iminothiolane, a method wherein various thiolamines are bonded to active esters of monomethoxypolyoxy

ethylenecarboxylic acid, and a method wherein a polyethylene glycol triazine derivative is bonded to thiolamine.

20 More specifically, as shown in the following Example, 2,4-bis(polyethylene glycol)-6-chloro-s-triazine (activated PEGII, manufactured by Seikagaku Kogyo K.K.) is reacted with cysteine, followed by reduction to obtain a cysteine-bonded activated PEGII.

21 (c) Supporting the Thiol-Modified Protein and the Compound Containing the Thiol-Modified Polyalkylene Glycol Moiety on the Surface of the Liposome

22 To bond the thiol-modified protein and the compound containing the thiol-modified polyalkylene glycol moiety to the surface of the liposome, firstly, the thiol-modified protein is added and reacted in a neutral buffer solution to the liposome having an excess amount of maleimide groups. For example, in the case of a thiol-modified antibody, the thiol-modified antibody is employed in an amount of from 0.1 mol to 20 mol per mol of maleimide groups. Then, to the remaining maleimide groups, an excess amount of the thiol-modified polyalkylene glycol, preferably in an amount of at least twice in equivalent, is added to obtain an antibody-bonded polyalkylene glycol-modified liposome. By this process, it is possible to accomplish the blocking effects of excess remaining maleimide groups.

23 (d) Method of Use of the Drug-Containing Protein-Bonded Liposome

24 The drug-containing protein-bonded liposome thus obtained, such as an adriamycin-containing antibody-bonded PEG-modified liposome, may be formulated into a form by a conventional method such as a lyophilization method (Japanese PCT Publication No. 503634/1990), a method wherein a stabilizer is added to form a liquid formulation (Japanese Unexamined Patent Publication No. 501116/1988) or a freeze-drying method (Japanese Unexamined Patent Publication No. 9961/1989).

25 The drug thus formulated can be used by e.g. intravascular administration or local administration such as intravesical or intraperitoneal administration against various diseases including cancer. The administration amount may be optionally selected depending on the drug contained in liposome.

26 In the case of an adriamycin-containing liposome, as an example, the dose is usually at most 50 mg/kg, preferably at most 10 mg/kg, more preferably at most 5 mg/kg, as the amount of adriamycin.

27 Now, the present invention will be described in further detail with reference to Examples. However, it should be understood that the present invention is by no means restricted to such specific Examples.

28 EXAMPLE 1

29 Confirmation of the Effect Whereby PEG-Modified Adriamycin-Containing Liposome Avoids the Reticuloendothelial System

30 Preparation of Thiol-Modified Polyethylene Glycol

31 45 mg of L-cysteine was dissolved in a 0.4M boric acid buffer solution. Then, 270 mg of 2,4-bis(polyethyleneglycol)-6-chloro-s-triazine (activated PEGII, manufactured by Seikagaku Kogyo K.K.) was added thereto, and the mixture was reacted at room temperature overnight. To the cysteine-bonded PEG thus obtained, 45 mg of dithiothreitol (DTT) was added, and the mixture was reacted at 37 degree. C. for 4 hours to obtain a solution containing a cysteine-bonded PEG. The reaction solution was further desalted by gel filtration on G-25 column (manufactured by Seikagaku Kogyo K.K.), and the solvent was replaced by 10 mM phosphate buffer solution pH7.4 and 0.15M NaCl (PBS). Then, the solution was added to 7 ml of thiopropylsepharose 6B (Pharmacia) equilibrated with PBS. Non-bonded substance was removed by washing with PBS. The cysteine-bonded PEG

bonded to the gel was eluted with PBS containing 50 mM DTT. Then, excess DTT was removed by gel filtration to obtain the above-identified product.

32 Preparation of Maleimide-Modified Dipalmitoylphosphatidyl Ethanolamine

33 117 mg of dipalmitoylphosphatidyl ethanolamine, 80 mg of N-(.epsilon.-maleimidocaproyloxy) succinimide (EMCS) and 44 .mu.l of triethylamine were added to a chloroform solution containing 1/3 of methanol and reacted under a nitrogen stream. Three hours later, 30 mg of EMCS was further added, and the mixture was further reacted at room temperature for 3 hours.

34 After confirming that the ninhydrin reaction of the reaction solution became negative, the reaction solution was evaporated to dryness under reduced pressure, and the product was dissolved again in a small amount of chloroform.

35 The maleimide-modified dipalmitoylphosphatidyl ethanolamine was purified by chromatography using WAKOSIL (manufactured by Gasshro Kogyo K.K.). Namely, the product was added to the column equilibrated with chloroform and developed with an eluting solution of chloroform/methanol=10/1 to obtain the desired substance.

36 Preparation of Maleimide-Containing Adriamycin-Loaded Liposome

37 1 ml of a 0.3M citric acid buffer solution pH4 was added to 100 mg of a solid lipid mixture of DSPC/chol-DPPA-maleimide-modified DPPE (3:10:2:0.5 (mol ratio) manufactured by Nippon Seika K.K.), and the mixture was stirred. Then, freezing-thawing was repeated five times for hydration to obtain a multi-lamella liposome. Then, the multi-lamella liposome was subjected to press-filtration ten times while heating at 60.degree. C. by a press apparatus (extruder, manufactured by Lipex Biomembranes) provided with a polycarbonate membrane having a pore size of 200 nm (nucleopore, Microscience), to obtain a liposome having a regulated particle size. This liposome solution was neutralized with a 1M NaOH solution. While heating the liposome solution at 60.degree. C., adriamycin (manufactured by Kyowa Hakko) was added in an amount of 1/10 by weight of the lipid. At least 97% of adriamycin was actively loaded to the liposome in accordance with the pH gradient between the interior and exterior of the liposome, to obtain a maleimide-containing adriamycin loaded liposome.

38 Introduction of Thiol-Modified PEG to the Maleimide-Containing Liposome

39 To the above maleimide-containing liposome, 5 .mu.mol of thiol-modified PEG was added, and the mixture was reacted in PBS at room temperature for 4 hours to obtain a PEG-modified adriamycin-loaded liposome. Further, the liposome was subjected to gel filtration by sepharose CL4B (pharmacia) to separate unreacted cysteine-bonded PEG, followed by evaluation test.

40 Study of Intracorporeal Behavior

41 The prepared liposome was intravenously administered to a mouse from the tail in an amount of 5 mg/kg as adriamycin, and 30 minutes later, the mouse was killed, and adriamycin in each of the extracted organs was extracted and quantitatively analysed in accordance with the method of Konno et al.

42 Namely, each organ was homogenized in a 0.3M hydrochloric acid, 90% ethanol, heated and centrifugally separated, whereupon the supernatant was measured by fluorescence of Ex 480 nm and Em 590 nm.

43 As shown in Table 1, a decrease in the amount of adriamycin in the liver and spleen was observed, and maintenance of a high concentration in the blood was observed.

TABLE 1

Concentration of adriamycin in the respective organs upon expiration of 30 minutes from the administration (ug/g tissue)

	free ADR	lip = ADR	PEG-lip = ADR
Blood	0.2	3.7	17.8
Liver	18.2	31.8	17.2
Spleen	7.2	113.1	90.4
Lung	7.9	4.2	3.5
Heart	3.2	1.8	3.5
Red	1.4	0.3	0.5
Brain	0	0.1	0.3

44 EXAMPLE 2

45 Confirmation of the Reactivity of the Antibody-Bonded PEG-Modified Liposome

46 1 ml of a 0.1M 6-carboxyfluorescein as a fluorescent marker was added to 100 mg of a solid lipid mixture (manufactured by Nippon Seika) of DPPC/chol/maleimide-modified DPPE=13/10/7.4 (mol ratio), and the mixture was hydrated and the particle size adjusted in the same manner as in Example 1 to obtain a maleimide-containing fluorescent dye-loaded liposome.

47 Preparation of a Thiol-Modified Antibody

48 To an antitumor mouse monoclonal antibody (19G), 1/40 mol amount of pepsin (Cooper Biomedical) in 0.1M acetic acid buffer solution pH 3.5, was added, and the mixture was reacted at 37.degree. C. overnight for digestion to obtain F(ab').sub.2. Further, by chromatography separation with a cationic exchange resin (mono S, manufactured by Pharmacia), F(ab' .sub.2 was isolated. The separation was conducted by a linear gradient of from 0M to 1.0M NaCl in a 0.1M acetic acid buffer solution pH4.0.

49 To reduce it to Fab', 11 .mu.l of 1% DTT was added per mg of the antibody in a 0.1M acetic acid buffer solution containing 0.15M NaCl (pH 4.5), and the mixture was left to stand at room temperature for 60 minutes. After completion of the reaction, demineralization was conducted by gel filtration on PD-10 column (manufactured by Pharmacia) equilibrated with PBS to obtain Fab'. To the liposome obtained from 100 mg of the above lipid, 5 mg of Fab' was added, and the mixture was reacted at 37.degree. C. for 5 hours and further 5 umol of thiol-modified polyethylene glycol was added to react it with excess maleimide, to obtain an antibody-bonded PEG-modified liposome.

50 Confirmation of the Bonding Activity of the PEG-Modified Antibody-Bonded Liposome

51 Using human gastoric cancer cell line MKN 45, of which the reactivity of the used monoclonal antibody had been confirmed, the reactivity of the PEG-modified antibody bonded liposome was confirmed in vitri.

52 The above carboxyfluorescein-baised antibody-bonded PEG-modified liposome was added to 5.times.10.sup.5 cells of MKN 45 floated in trypsin, and the mixture was reacted in 90% human inactivated serum at 37.degree. C. for 240 minutes. The centrifugal pellet of cells was washed with PBS, and then carboxyfluorescein was freed at 60.degree. C. with 10% triton.times.100, and the amount bonded to the cells was calculated by a fluorescence measurement.

53 As shown in FIG. 1, a high reactivity with the objective cells was observed

also in the case of the antibody-bonded PEG-modified liposome.

54 EXAMPLE 3

55 Confirmation of the Pharmacological Activities of the Adriamycin-Loaded Monoclonal Antibody-Bonded PEG-Modified Liposome

56 A solid lipid mixture of DPPC:cholesterol:maleimide-modified DPPE-PEG-10/0.5 (mol ratio) was treated in the same manner as in Example 1 to obtain an adriamycin-loaded maleimide-containing liposome.

57 Using a human monoclonal antibody (IgG), Fab'-modified antibody was obtained in the same manner as in Example 2 except that the pH for the pepsin digestion was changed to 4.0, and it was subjected to the bonding with the liposome. Further, it was modified by thiol-modified PEG in the same manner to obtain an adriamycin-loaded human monoclonal antibody-bonded PEG-modified liposome.

58 Evaluation of the Pharmacological Activities Using A Human Gastric Cancer Cell Line-Transplanted Nude Mouse System

59 Using human cancer cell line MKN 45 of which the reactivity with the antibody was observed in vitro, and accumulation was observed in vivo with respect to the nude mouse-transplanted system, the antitumor activities were studied.

60 For a therapeutic test, 1.times.10.sup.6 cells of MKN 45 cultured, were subcutaneously transplanted to a nude mouse, and the therapeutic test was initiated when the weight of the tumor became about 100 mg ten days later. On the first day, the fourth day and the ninth day from the initiation of the therapy, the liposome was intravenously administered to the mouse from the tail in an amount of 5 mg/kg as adriamycin. To measure the change with time of the proliferation of the tumor, an assumed tumor weight was obtained by a calculation formula of short diameter.times.short diameter.times.long diameter/2 of the tumor in accordance with a Battelle Columbus method, and the change with time was shown using as a reference the weight of the tumor at the initiation of the therapy.

61 As a result, as shown in FIG. , strong antitumor activities of the adriamycin-loaded monoclonal antibody-bonded PEG-modified liposome were shown.

62 With the liposome obtained by the present invention, it is possible to suppress the non-specific capture in the reticuloendothelial system such as liver or spleen as observed with the conventional liposomes, and thus the liposome of the present invention is effective for use as a selective chemotherapeutic drug, particularly as a cancer treating drug.

CLAIMS:

We claim:

1. A drug containing, protein-bonded liposome, which comprises a liposome containing a drug, said liposome having maleimide groups on the surface thereof, wherein a portion of the maleimide groups are bonded to a thiol group-containing protein and a remaining portion of the maleimide groups are bonded to a thiol group-containing polyethylene glycol moiety; said liposome comprising phosphatidyl choline, cholesterol and maleimide-modified phosphatidyl ethanolamine.

2. The drug-containing, protein-bonded liposome of claim 1, wherein the protein is selected from the group consisting of an antibody, FGF and EGF.

3. The drug-containing, protein-bonded liposome of claim 2, wherein said protein is an antibody selected from the group consisting of animal polyclonal antibodies, mouse monoclonal antibodies, human-mouse chimeric antibodies and

human monoclonal antibodies.

4. The drug-containing, protein-bonded liposome of claim 3, wherein said protein is a human monoclonal antibody.

5. The drug-containing, protein-bonded liposome of claim 1, wherein the liposome comprises dipalmitoylphosphatidyl choline, cholesterol and maleimide-modified dipalmitoylphosphatidyl ethanolamine.

6. The drug-containing, protein-bonded liposome of claim 5, wherein the maleimide-modified dipalmitoylphosphatidyl ethanolamine is obtained by reacting N-(.epsilon.-maleimidocaproyloxy) succinimide and dipalmitoylphosphatidyl ethanolamine.

7. The drug-containing, protein-bonded liposome of claim 1, wherein the thiol group-containing protein having maleimide groups bonded thereto is obtained by reacting maleimide residues on the liposome surface and a thiol group-containing protein.

8. The drug-containing, protein-bonded liposome of claim 1, wherein said thiol-group containing polyethylene glycol moiety having maleimide groups bonded thereto is obtained by reacting maleimide residues on the liposome surface and a thiol-group containing polyethylene glycol.

9. The drug-containing, protein-bonded liposome of claim 1, wherein said drug comprises an antitumor drug, a .beta.-lactam antibiotic, a toxin, an aminoglycoside, antisense RNA or actinoplane.

10. The drug-containing, protein-bonded liposome of claim 9, wherein the antitumor drug is selected from the group consisting of adrianycin, daunomycin, mitomycin, cisplatin, vincristine, epirubicin, methotrexate, 5-FU and aclacinomycin.

11. The drug containing, protein-bonded liposome of claim 9, wherein said aminoglucoside is gentamicin.

12. The drug containing, protein-bonded liposome of claim 9, wherein said .beta.-lactam antibiotic is sulpenisillin.

13. The drug containing, protein-bonded liposome of claim 9, wherein said toxin is ricin A or diphtheria toxin.

14. The drug containing, protein-bonded liposome of claim 9, wherein said antisense RNA is antisense RNA against HIV or ras gene.

15. An antitumor drug, comprising a liposome containing a drug, said liposome having maleimide groups on the surface thereof, wherein a portion of the maleimide groups are bonded to a thiol group-containing protein and a remaining portion of the maleimide groups are bonded to a thiol group-containing polyethylene glucol moiety; said liposome comprising phosphatidyl choline, cholesterol and maleimide-modified phosphatidyl ehtanolamine.